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Data access for the 1,000 Plants (1KP) project

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Abstract

The 1,000 plants (1KP) project is an international multi-disciplinary consortium that has generated transcriptome data from over 1,000 plant species, with exemplars for all of the major lineages across the *Viridiplantae* (green plants) clade. Here, we describe how to access the data used in a phylogenomics analysis of the first 85 species, and how to visualize our gene and species trees. Users can develop computational pipelines to analyse these data, in conjunction with data of their own that they can upload. Computationally estimated protein-protein interactions and biochemical pathways can be visualized at another site. Finally, we comment on our future plans and how they fit within this scalable system for the dissemination, visualization, and analysis of large multi-species data sets.

Keywords: *Viridiplantae*, Biodiversity, Transcriptomes, Phylogenomics, Interactions, Pathways

Introduction

The 1,000 plants (1KP) project is an international multi-disciplinary consortium that has now generated transcriptome data from over 1,000 plant species. One of the goals of our species selection process was to provide exemplars for all of the major lineages across the *Viridiplantae* (green plants), representing approximately one billion years of evolution, including flowering plants, conifers, ferns, mosses and streptophyte green algae. Whereas genomics has long strived for completeness within species (e.g., every gene in the species), we were focused on completeness across an evolutionary clade – obviously not every species, but one representative species for everything at some phylogenetic level (e.g., one species per family, and perhaps more than one species when the family is especially large). Because many of our species had never been subjected to large-scale sequencing, 2 gigabases (Gb) of data per sample was sufficient to increase the number of plant genes by approximately 100-fold in comparison to the totality of the public databases.

The 1KP project began as a public-private partnership, with 75% of the funding provided by the Government of Alberta and 25% by Musea Ventures. Significant in-kind contributions were provided by BGI-Shenzhen in the form of reduced sequencing costs and by the NSF-funded iPlant collaborative [1] in the form of computational informatics support. Many plant scientists from around the world were involved in the collection of live tissue samples and in the extraction of RNA. Additional computing resources were provided by Compute Canada and by the China National GeneBank. Despite the constraints of this funding model, we released our data (on a collaborative basis) to scientists who approached us with goals that did not compete with ours. For the general community, access was provided through a BLAST portal [2].

We believed that there would be intrinsic value in data of this nature that is beyond our imagination. But for the initial publication, we agreed on two objectives. Firstly, by adopting a phylogenomics approach we hoped to resolve many of the lingering uncertainties in species

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relationships, especially in the early lineages of streptophyte green algae and land plants, where previous analyses were based on comparatively sparse taxonomic densities. And secondly, despite the limitations of these data, we hoped to identify some of the gene changes associated with the major innovations in *Viridiplantae* evolution, such as multicellularity, transitions from marine to freshwater or terrestrial environments, maternal retention of zygotes and embryos, complex life history involving haploid and diploid phases, vascular systems, seeds and flowers.

Our RNA extraction protocols [3] and our RNA-Seq transcriptome assembly algorithms [4] have already been published. Here, we are publishing the second of two linked papers. The first is a review of the state-of-knowledge for *Viridiplantae* species relationships and our initial foray into the phylogenomics on a subset of 1KP [5]. The other is a description of the websites that we created in order to provide access to the data (from raw reads to computed results), visualize the results, and perform custom analyses in conjunction with external data that the users can upload. An initial gene annotation is also provided, which focuses on the functional relationships between proteins and their associated metabolites.

Review

Access to raw and processed data

Our initial phylogenomics effort used sequences from multiple sources. They include transcriptomes from 1KP representing 85 species, transcriptomes from other sources representing 7 species, and genomes representing an additional 11 species. A summary of these data sources is given in Table 1. We submitted all of the unassembled reads from the 1KP transcriptomes to the Short Reads Archive (SRA) under project accession PRJEB4921 "1000 Plant (1KP) Transcriptome: The Pilot Study." Note that, with the exception of *Eschscholzia californica*, we sequenced only one sample per species.

To make it easier for others to reproduce our phylogenomics analyses, we are releasing our intermediate computations, not just the final results. Everything is hosted at the iPlant Data Store, a high performance, large capacity, distributed storage system. The contents include transcriptome assemblies, putative coding sequences, orthogroups (i.e., from the 11 reference genomes), as well as gene and species trees with related sequence alignments. There are quite a lot of files and their total sizes are not negligible; so before users begin to download these files, we suggest that they consult Table 2 for a description of what to expect.

At the simplest level, anonymous downloads are permitted from a designated area of the iPlant Data Store [6]. However, much greater functionality is available through the iPlant resources that we describe in the following sections.

Visualization and custom analyses

To take full advantage of the iPlant computational infrastructure, it is necessary to first register at [7]. Accounts are free, and in addition to 1KP data, users will find high performance computing and cloud-based services. Multiple access modalities are supported: anonymous and secure web interfaces, desktop clients and high-speed command lines. However, we feel that for most users the best option is the iPlant discovery environment (DE), a web-based interface that provides users with high-performance computing resources and data storage. Most contemporary web browsers are supported, including Safari v. 6.1, Firefox v. 24, and Chrome v. 34. The caveat is that some of these functionalities (see below) require Java 1.6.

To guide users through its resources, iPlant is constantly producing new tutorials and teaching materials, including live and recorded webinars. The full catalog can be found at [8]. Here, we describe the new resources specifically created for 1KP.

Discovery environment (DE)

For access to the 1KP files, users should visit [9] and search for a folder called *Community Data/onekp_pilot* Figure 1.

From the data window it is possible to download individual files or perform bulk downloads of multiple files and directories through a Java plugin. Note that for security reasons, some operating systems will not allow users to run Java applets. In this instance, a window will pop up to tell the user that there is a problem, and the user should follow the instructions that are given to configure an iDrop desktop [10] Figure 2.

It is possible to perform analyses directly in the DE using any of the 1KP files as input; for example, users can re-compute the sequence alignments and gene trees using different algorithms and parameters [11] Figure 3. More generally, users can select from a variety of applications in the Apps catalogue, which is constantly growing, and includes many popular bioinformatics tools for large-scale phylogenetics, genome-wide associations and next generation sequence analyses.

Species and gene trees can be explored with the iPlant tree viewer, *Phylozoom*, a newly developed web-based phylogenetic tree viewer that supports trees with hundreds of thousand leaves and allows for semantic zooming Figure 4. To access the tree viewer, users need only click on a tree file. This will open a preview window with two tabs: one for the tree's newick string (a format for graph-theoretical trees as defined at [12]) and another for the web link that opens a window to the tree display. Notice that pop-ups must be enabled on the user's browser.

To zoom in and expand the collapsed clades, click on the node of interest. To zoom out, click and drag the tree

Table 1 Data sources for phylogenomics analyses

Species	Type	Accession	iPlant ID
Arabidopsis thaliana	genome	n/a	n/a
Brachypodium distachyon	genome	n/a	n/a
Carica papaya	genome	n/a	n/a
Medicago truncatula	genome	n/a	n/a
Oryza sativa	genome	n/a	n/a
Physcomitrella patens	genome	n/a	n/a
Populus trichocarpa	genome	n/a	n/a
Selaginella moellendorffii	genome	n/a	n/a
Sorghum bicolor	genome	n/a	n/a
Vitis vinifera	genome	n/a	n/a
Zea mays	genome	n/a	n/a
Aquilegia formosa	meta-assembly	PlantGDB	AQUI
Cycas rumphii	meta-assembly	SRX022306, SRX022215	CYCA
Liriodendron tulipifera	meta-assembly	PRJNA46857	LIRI
Persea americana	meta-assembly	PRJNA46857	PERS
Pinus taeda	meta-assembly	PRJNA79733	PINU
Pteridium aquilinum	meta-assembly	PRJNA48473	PTER
Zamia vazquezii	meta-assembly	PRJNA46857	ZAMI
Acorus americanus	OneKP meta-assembly	ERR364395, PRJNA46857	ACOR
Amborella trichopoda	OneKP meta-assembly	ERR364329, PRJNA46857	AMBO
Catharanthus roseus	OneKP meta-assembly	ERR364390, PRJNA79951, PRJNA236160	CATH
Eschscholzia californica	OneKP meta-assembly	ERR364338, ERR364335, ERR364336, ERR364337, ERR364334, SRX002988, SRX002987, PlantGDB	ESCH
Ginkgo biloba	OneKP meta-assembly	ERR364401, PlantGDB	GINK
Nuphar advena	OneKP meta-assembly	ERR364330, PRJNA46857	NUPH
Ophioglossum petiolatum	OneKP meta-assembly	ERR364410, SRX666586	OPHI
Saruma henryi	OneKP meta-assembly	ERR364383, PRJNA46857	SARU
Welwitschia mirabilis	OneKP meta-assembly	ERR364404, PRJNA46857	WELW
Allamanda cathartica	OneKP	ERR364389	MGVU
Angiopteris evecta	OneKP	ERR364409	NHCM
Anomodon attenuatus	OneKP	ERR364349	QMWB
Bazzania trilobata	OneKP	ERR364415	WZYK
Boehmeria nivea	OneKP	ERR364387	ACFP
Bryum argenteum	OneKP	ERR364348	JMXW
Cedrus libani	OneKP	ERR364342	GGEA
Ceratodon purpureus	OneKP	ERR364350	FFPD
Chaetosphaeridium globosum	OneKP	ERR364369	DRGY
Chara vulgaris	OneKP	ERR364366	CHAR
Chlorokybus atmophyticus	OneKP	ERR364371	AZZW
Colchicum autumnale	OneKP	ERR364397	NHIX
Coleochaete irregularis	OneKP	ERR364367	QPDY
Coleochaete scutata	OneKP	ERR364368	VQBJ
Cosmarium ochthodes	OneKP	ERR364376	STKJ
Cunninghamia lanceolata	OneKP	ERR364340	OUOI

Table 1 Data sources for phylogenomics analyses *(Continued)*

Cyathea (Alsophila) spinulosa	OneKP	ERR364412	GANB
Cycas micholitzii	OneKP	ERR364405	XZUY
Cylindrocystis brebissonii	OneKP	ERR364378	YOXI
Cylindrocystis cushleckae	OneKP	ERR364373	JOJQ
Dendrolycopodium obscurum	OneKP	ERR364346	XNXF
Dioscorea villosa	OneKP	ERR364396	OCWZ
Diospyros malabarica	OneKP	ERR364339	KVFU
Entransia fimbriata	OneKP	ERR364372	BFIK
Ephedra sinica	OneKP	ERR364402	VDAO
Equisetum diffusum	OneKP	ERR364408	CAPN
Gnetum montanum	OneKP	ERR364403	GTHK
Hedwigia ciliata	OneKP	ERR364352	YWNF
Hibiscus cannabinus	OneKP	ERR364388	OLXF
Houttuynia cordata	OneKP	ERR364332	CSSK
Huperzia squarrosa	OneKP	ERR364407	GAON
Inula helenium	OneKP	ERR364393	AFQQ
Ipomoea purpurea	OneKP	ERR364392	VXKB
Juniperus scopulorum	OneKP	ERR364341	XMGP
Kadsura heteroclita	OneKP	ERR364331	NWMY
Klebsormidium subtile	OneKP	ERR364370	FQLP
Kochia scoparia	OneKP	ERR364385	WGET
Larrea tridentata	OneKP	ERR364386	UDUT
Leucodon brachypus	OneKP	ERR364353	ZACW
Marchantia emarginata	OneKP	ERR364417	TFYI
Marchantia polymorpha	OneKP	ERR364416	JPYU
Mesostigma viride	OneKP	ERR364365	KYIO
Mesotaenium endlicherianum	OneKP	ERR364377	WDCW
Metzgeria crassipilis	OneKP	ERR364359	NRWZ
Monomastix opisthostigma	OneKP	ERR364362	BTFM
Mougeotia sp.	OneKP	ERR364374	ZRMT
Nephroselmis pyriformis	OneKP	ERR364363	ISIM
Netrium digitus	OneKP	ERR364379	FFGR
Nothoceros aenigmaticus	OneKP	ERR364356	DXOU
Nothoceros vincentianus	OneKP	ERR364357	TCBC
Penium margaritaceum	OneKP	ERR364382	AEKF
Podophyllum peltatum	OneKP	ERR364384	WFBF
Polytrichum commune	OneKP	ERR364413	SZYG
Prumnopitys andina	OneKP	ERR364343	EGLZ
Pseudolycopodiella caroliniana	OneKP	ERR364345	UPMJ
Psilotum nudum	OneKP	ERR364411	QVMR
Pyramimonas parkeae	OneKP	ERR364361	TNAW
Rhynchostegium serrulatum	OneKP	ERR364355	JADL
Ricciocarpos natans	OneKP	ERR364358	WJLO
Rosmarinus officinalis	OneKP	ERR364391	FDMM
Rosulabryum cf. *capillare*	OneKP	ERR364351	XWHK

Table 1 Data sources for phylogenomics analyses *(Continued)*

Roya obtusa	OneKP	ERR364380	XRTZ
Sabal bermudana	OneKP	ERR364400	HWUP
Sarcandra glabra	OneKP	ERR364333	OSHQ
Sciadopitys verticillata	OneKP	ERR364344	YFZK
Selaginella stauntoniana	OneKP	ERR364347	ZZOL
Smilax bona-nox	OneKP	ERR364398	MWYQ
Sphaerocarpos texanus	OneKP	ERR364360	HERT
Sphagnum lescurii	OneKP	ERR364414	GOWD
Spirogyra sp.	OneKP	ERR364375	HAOX
Spirotaenia minuta	OneKP	ERR364381	NNHQ
Tanacetum parthenium	OneKP	ERR364394	DUQG
Taxus baccata	OneKP	ERR364406	WWSS
Thuidium delicatulum	OneKP	ERR364354	EEMJ
Uronema sp.	OneKP	ERR364364	ISGT
Yucca filamentosa	OneKP	ERR364399	ICNN

Meta-assembly refers to a transcriptome assembled from more than one sequenced sample. Some of these were a combination of 1KP and other data; some were entirely non-1KP. Accession numbers (SRA or otherwise) are given for all of the transcriptomes that we used.

figure to the left. To zoom out completely, click the space bar. The web address is a unique identifier that can be shared with others to let them to visualize the tree.

For more advanced users wanting to perform more complicated procedures, iPlant capabilities are available from a command line. It is based on the integrated rule-oriented data system (iRODS) [13]. All the user has to do is install a command line utility, *icommands*, which mimics UNIX and enables high-speed parallel data transfers. Instructions are available at [14].

Interactions and pathways

In addition to the tree-based species and gene relationships at the iPlant site, functional relationships between proteins and their associated metabolites are available from the Computational Biology Group at the University of Washington, developers of CANDO [15]. Sequence similarity-based methods are used to map 1KP proteins to curated repositories of protein-protein interactions (i.e., BioGRID [16]) and biochemical pathways (i.e., Kyoto Encylopedia of Genes and Genomes [KEGG] [17]). The user can select any metabolic pathway defined by KEGG and, within this context, see all the 1KP proteins from their chosen species, with functional annotations inferred from KEGG. This website is at [18] Figure 5.

Note that, over the course of this project, there have been many improvements in the transcriptome assemblies. The phylogenomics work (now being published) was done with the SOAPdenovo algorithm. A second assembly was subsequently done with the newer SOAPdenovo-trans algorithm, which we incorporated into the newer interactions and pathways work. However, both sets of assemblies are available through the iPlant data store.

Conclusions

The rest of the 1KP data will be released, on much the same platform, along with our analyses of all one thousand species. Our scientific objectives are given at [19]. We have always been open about our intentions, because we wanted to avoid conflict among the scientists who were already working with 1KP and offer early pre-publication access to other non-competing scientists. As soon as we see a draft of a paper, we track its progress through the review process at [20]. Some of these papers have already been published, and more than a few required years of follow-up experiments, resulting for example in fundamental discoveries for molecular evolution [21] and (surprisingly) new tools for mammalian neurosciences [22].

Many of these studies were not anticipated when 1KP was conceived. We only knew that, just as there was value in sequencing every gene in a genome, despite not knowing *a priori* what many of the genes might do, there would be value in sequencing across an ancient and ecologically dominant clade, even when many of the species have no obvious economic or scientific value that would justify a genome sequencing effort. Transcriptomes were a less expensive way to explore plant diversity, and demonstrate value beyond the obvious species.

Table 2 Number and size of data files on websites

File count	Median size (Mb)	Average size (Mb)	Largest size (Mb)	Total size (Mb)	Similar directories	iPlant directory name
68,253	0.0	0.3	481.1	23,116.6		onekp_pilot
48,053	0.0	0.3	481.1	14,956.7		onekp_pilot/orthogroups
19,220	0.1	0.7	243.8	13,276.5		onekp_pilot/orthogroups/alignments
9,610	0.1	0.3	79.8	3,289.6		onekp_pilot/orthogroups/alignments/FAA
9,610	0.2	1.0	243.8	9,986.9		onekp_pilot/orthogroups/alignments/FNA
28,833	0.0	0.1	481.1	1,680.2		onekp_pilot/orthogroups/gene_trees
9,611	0.0	0.1	481.1	583.3		onekp_pilot/orthogroups/gene_trees/FAA
9,610	0.0	0.0	0.5	102.2		onekp_pilot/orthogroups/gene_trees/FAA/trees
19,222	0.0	0.1	458.0	1,096.8		onekp_pilot/orthogroups/gene_trees/FNA
9,611	0.0	0.1	458.0	556.6		onekp_pilot/orthogroups/gene_trees/FNA/12_codon
9,610	0.0	0.0	0.5	98.5		onekp_pilot/orthogroups/gene_trees/FNA/12_codon/trees
9,611	0.0	0.1	439.1	540.3		onekp_pilot/orthogroups/gene_trees/FNA/all_codon
9,610	0.0	0.0	0.5	101.2		onekp_pilot/orthogroups/gene_trees/FNA/all_codon/dna_tree
19,919	0.0	0.2	175.2	3,468.8		onekp_pilot/phylogenetic_analysis
2,556	0.1	0.1	1.0	292.7		onekp_pilot/phylogenetic_analysis/alignments
852	0.0	0.0	0.3	41.8		onekp_pilot/phylogenetic_analysis/alignments/FAA
852	0.1	0.1	1.0	125.5		onekp_pilot/phylogenetic_analysis/alignments/FNA
852	0.1	0.1	0.9	125.4		onekp_pilot/phylogenetic_analysis/alignments/FNA2AA
17,197	0.0	0.1	0.4	1,827.3		onekp_pilot/phylogenetic_analysis/gene_trees
1,704	0.0	0.1	0.4	238.3		onekp_pilot/phylogenetic_analysis/gene_trees/FAA
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/FAA/raxmlboot.####
1,704	0.0	0.1	0.4	238.3		onekp_pilot/phylogenetic_analysis/gene_trees/FNA
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/FNA/raxmlboot.####
3,408	0.0	0.1	0.4	476.7		onekp_pilot/phylogenetic_analysis/gene_trees/FNA2AA
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/FNA2AA/raxmlboot.####
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/FNA2AA/raxmlboot.####.c1c2
10,381	0.0	0.1	0.4	874.0		onekp_pilot/phylogenetic_analysis/gene_trees/filtered
2,548	0.0	0.1	0.4	169.3		onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FAA
1	0.0	0.0	0.0	0.0	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FAA/raxmlboot.####.f25
1	0.2	0.1	0.4	0.2	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FAA/raxmlboot.####.filterlen33
852	0.0	0.0	0.0	3.8		onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA
1	0.0	0.0	0.0	0.0	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA/raxmlboot.####.f25
6,980	0.0	0.1	0.4	700.9		onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.GAMMA.2
2	0.3	0.1	0.4	0.3	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.c1c2.GAMMA.2
1	0.0	0.0	0.0	0.0	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.c1c2.f25

Table 2 Number and size of data files on websites *(Continued)*

1	0.0	0.0	0.0	0.0	852	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.f25
2	0.2	0.1	0.4	0.2	844	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.filterlen33
1	0.3	0.3	0.4	0.3	180	onekp_pilot/phylogenetic_analysis/gene_trees/filtered/FNA2AA/raxmlboot.####.filtered25.GAMMA.2
166	0.0	8.1	175.2	1,348.8		onekp_pilot/phylogenetic_analysis/species_level
50	15.0	27.0	175.2	1,348.1		onekp_pilot/phylogenetic_analysis/species_level/alignments
15	14.7	14.3	58.3	214.2		onekp_pilot/phylogenetic_analysis/species_level/alignments/FAA
35	29.4	32.4	175.2	1,133.9		onekp_pilot/phylogenetic_analysis/species_level/alignments/FNA
116	0.0	0.0	0.0	0.6		onekp_pilot/phylogenetic_analysis/species_level/trees
276	10.0	17.0	157.4	4,691.1		onekp_pilot/taxa
3	9.7	17.0	157.4	51.0	92	onekp_pilot/taxa/####-############
1	30.8	17.0	157.4	36.0	92	onekp_pilot/taxa/####-############/assemblies
2	9.7	7.5	45.2	15.0	92	onekp_pilot/taxa/####-############/translations
5	0.0	0.0	0.0	0.1		onekp_pilot/tools
File count	**Median size (Mb)**	**Average size (Mb)**	**Largest size (Mb)**	**Total size (Mb)**	**Similar directories**	**Contents at SRA (PRJEB4921)**
178	1,915.0	2,045.5	3,371.0	364,100.0		total of all short reads – uncompressed, but downloads are compressed to a quarter of these sizes
2	1,915.0	2,045.5	3,371.0	4,091.0	89	expecting per sample – uncompressed, but downloads are compressed to a quarter of these sizes

In some instances, users will find many directories with similar names, as indicated in this table by hash (#) marks. The total number of directories is given in the preceding column.

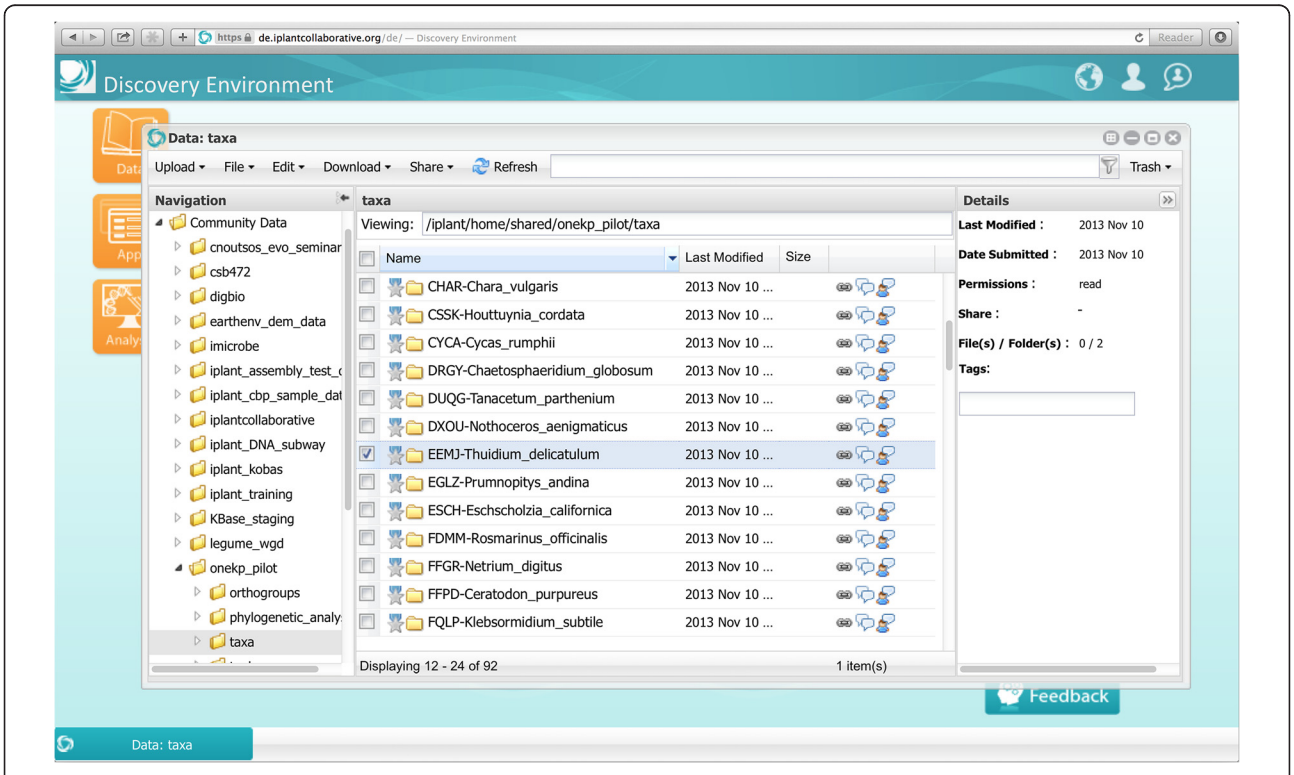

Figure 1 iPlant DE data window.

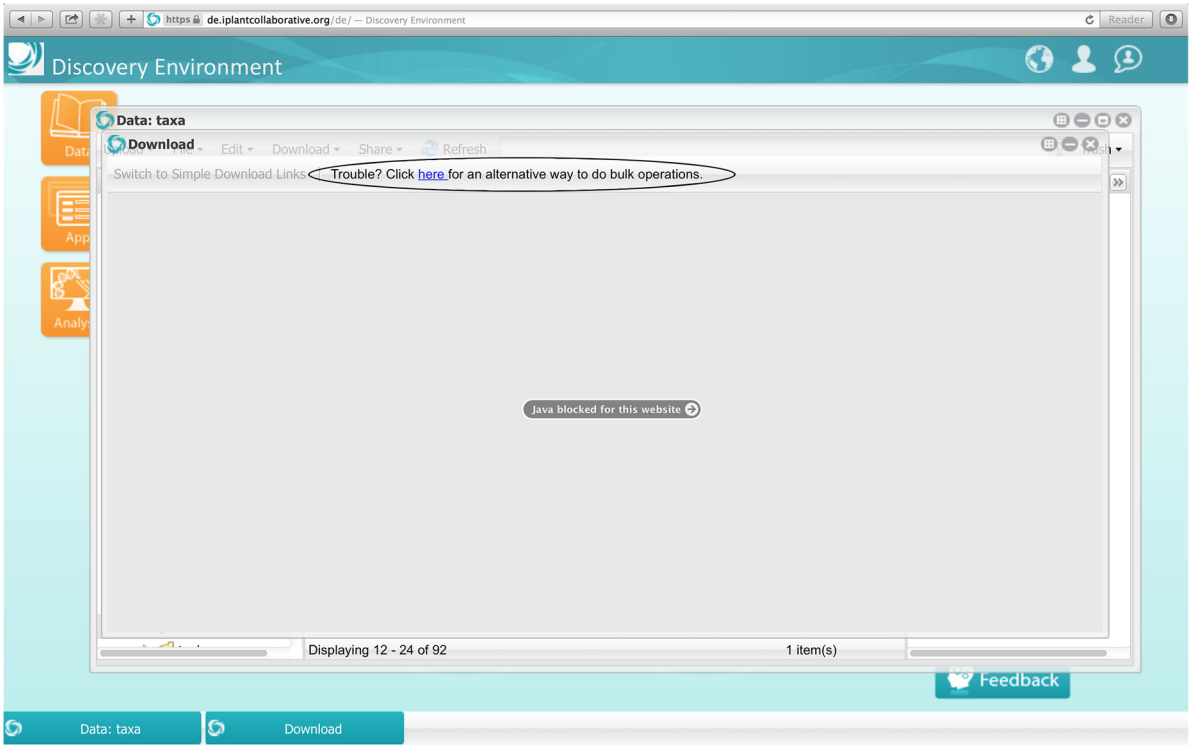

Figure 2 Bulk download window if Java is disabled. Click on the circled link to access the instructions to install and configure an iDrop desktop.

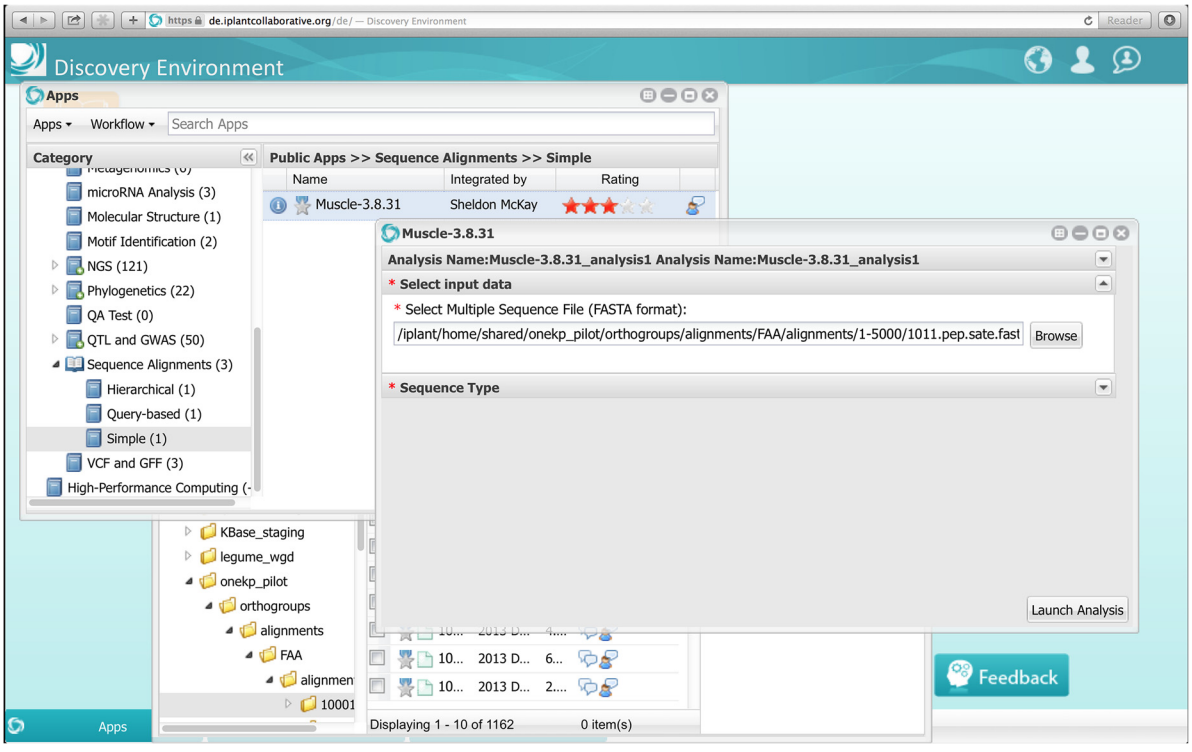

Figure 3 Realigning a group of sequences using *Muscle*.

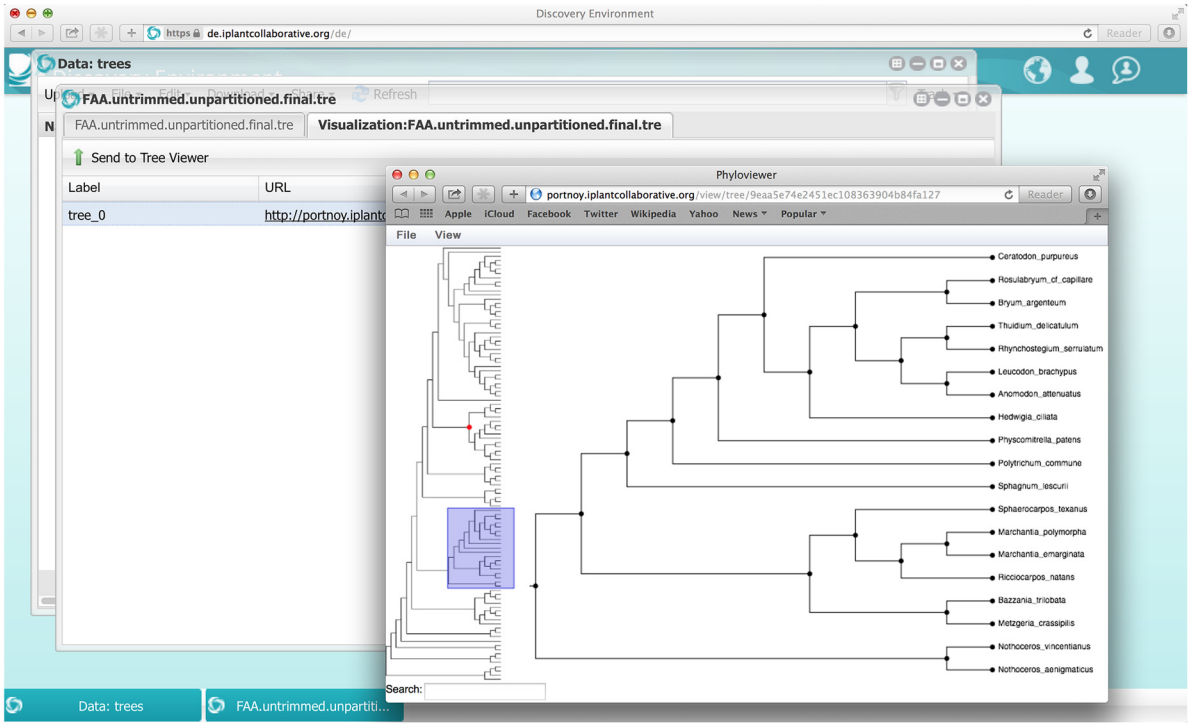

Figure 4 ***Phylozoom*** **display of 1KP species phylogeny.**

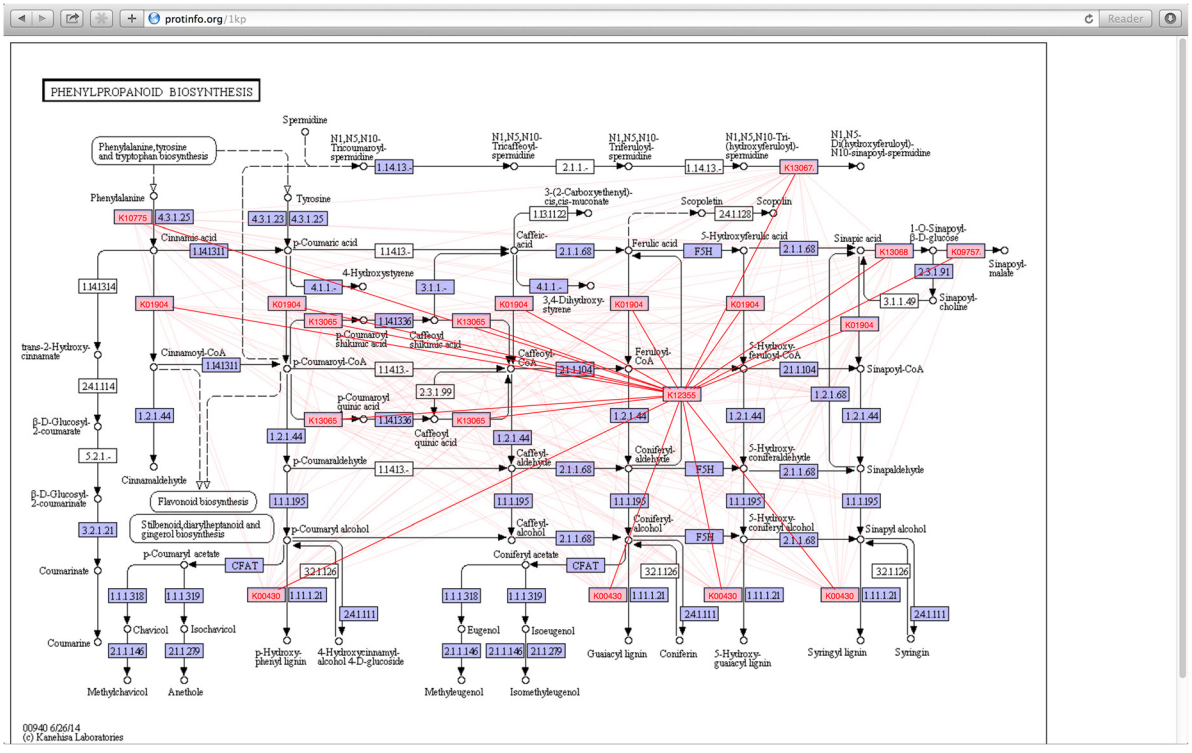

Figure 5 Phenylpropanoid synthesis pathway for *Colchicum autumnale*. Labelled rectangles are proteins. Small circles are metabolites. Black lines show the KEGG pathway. Red lines show the BioGRID interactions emanating from protein (K12355), which was interactively selected. A right-click on the protein will display the inferred function and a link to the sequence(s).

Abbreviations
1KP: 1,000 Plants project; DE: Discovery Environment; KEEG: Kyoto Encyclopedia of Genes and Genomes; NSF: National Science Foundation; SRA: Short Reads Archive.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
CWD, BRR, NWM, SWG, S Ma, BS, MM, DES, PSS, CR, LP, JAS, LD, DWS, JCV, TC, TMK, MR, RSB, MKD, and JLM collected the plant samples. NM, NJW, S Mi, NN, TW, SA, MB, JGB, MAG, EW, JPD, CWD, BR, HP, BRR, and JLM performed the phylogenomic analyses. NM, LHH, ZY, and EJC setup and maintained web-resources used to communicate data. LHH and RS performed the protein and KEGG pathway analyses. EJC, ZT, XW, XS, YZ, JW, and GKW generated the sequence data. GKW and JLM designed and oversaw the research. All authors read and approved the final manuscript.

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